Characterization and classification of mycorrhizae of Douglas fir. II. *Pseudotsuga menziesii* + *Rhizopogon vinicolor*

B. ZAK

Pacific Northwest Forest and Range Experiment Station, U.S. Department of Agriculture, Corvallis, Oregon

Received September 14, 1970

ZAK, B. 1971. Characterization and classification of mycorrhizae of Douglas fir. II. *Pseudotsuga menziesii* + *Rhizopogon vinicolor*. Can. J. Bot. **49**: 1079–1084.

A common tuberculate ectomycorrhiza of Douglas fir in the Pacific Northwest, described earlier by Trappe, is further examined and defined. Tubercles consist of an outer rind of aseptate, amber, thick-walled hyphae encasing tightly packed inner elements mantled with septate, hyaline, thin-walled hyphae. Reported as a Phycomycete and a Basidiomycete, respectively, the two hyphal forms actually belong to a single fungus, *Rhizopogon vinicolor* A. H. Smith; cultural characteristics of this fungus are described. Pure culture mycorrhiza syntheses with both mycorrhizal and sporocarpic isolates and Douglas-fir seedlings are reported. Antagonism tests revealed the following inhibition of root pathogens by *R. vinicolor*: strong—*Phytophthora cinnamomi* Rands, *Pythium debaryanum* Heese, and *Pythium sylvaticum* Campbell & Hendrix; moderate—*Fomes annosus* (Fr.) Cke. and *Poria weirii* Murr.; and weak or none—*Fusarium oxysporum* f. *pini* (Hartig) Snyd. & Hans., *Pythium ultimum* Trow, *Rhizoctonia solani* Kuehn, and *Macrophomina phaseoli* (Maubl.) Ashby.

Introduction

Trappe (1965) described a tuberculate mycorrhiza of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii* and var. *glauca* (Beissn.) Franco) occurring in western coastal states and British Columbia. Apparently the same mycorrhiza was briefly described earlier by Dominik (1963) and recently by Dominik and Majchrowicz (1967) in Poland. These workers implicated two distinct but unidentified fungi, one with septate and the other with aseptate hyphae, in formation of the mycorrhiza. Recent studies by the author, however, indicate both fungi to be one, *Rhizopogon vinicolor* A. H. Smith. This paper presents evidence of this association, further defines the mycorrhiza, now designated *P. menziesii* + *R. vinicolor*, and lists cultural characteristics of the fungal symbiont. It is one of a series characterizing and classifying Douglas-fir mycorrhizae. An earlier paper (Zak 1969*a*) described two distinct Douglas-fir mycorrhizae formed by two strains of *Poria terrestris* (DC. ex Fries) Sacc.

Occurrence and Development

The mycorrhiza is found in the upper soil layers and especially in decayed stumps, logs, and wood debris partially buried in the soil. It occurs in Douglas-fir stands of all ages and was also observed on roots of an approximately 8-month-old seedling growing atop a large decayed stump. However, it never has been found on seedlings in forest tree nurseries. Trappe (1965) lists the range as west of the Cascade Range from northern California to northern Vancouver Island for var. *menziesii*, and in the Ochoco Mountains, Oregon, and at Slocan Lake, British Columbia, east of the Cascades for var. *glauca*.

In western Oregon, where winter is mild, fresh mycorrhizae appear in October after the start of fall rains. They are best observed in the tops of large decayed stumps (0.6–2 m high and 0.9–2 m in diameter), growing in moist and friable debris. Last season's dried and dead mycorrhizae may be found under bark slabs along the yet powder-dry sides of the stump.

Other Douglas-fir mycorrhizae often associated along the same root with this mycorrhiza in decayed wood include *Pseudotsuga menziesii* + *Cenococcum graniforme*,[1] *P. menziesii* + *Poria terrestris* (blue-staining), *P. menziesii* + *P. terrestris* (orange-staining), *P. menziesii* + *P. terrestris* (rose-staining) (Zak 1969*b*), and two unidentified forms.

Gross Morphology

The mycorrhiza is tuberculate (Fig. 1A–C), the tubercles ranging from 2 or 3 mm to over 25 mm in diameter but usually 5–15 mm. Those formed in fissures in decayed wood are often flattened. The young, enlarging tubercle (Fig. 2A) is covered by a thin, loose outer veil of mycelium through which mycorrhizal elements are visible. Their protrusion gives the young tubercle a

[1] *Cenococcum graniforme* (Sow.) Ferd. & Winge.

knobby form. First silvery white, the outer covering becomes lightly and then completely vinaceous or a light to darker brown.

As the tubercle matures (Fig. 2B), the covering thickens into a firm, felty rind, changing to brown-black to black with blotches of greyish bloom. The surface becomes finely velvety and then smooth. Elements no longer protrude; in form, the tubercle resembles a tiny potato. The firm rind is easily peeled away to reveal the densely packed mycorrhizal elements (Fig. 1B). Internal structure consists of one or more deformed pyramidally pinnate fans (Fig. 1C) and usually originates from a single "short root" or rootlet. The elements are finely pubescent and silvery white to very pale pinkish brown.

Occasionally a rift may be observed in the rind of an enlarging mature tubercle. As the rind is stripped away and the elements pulled apart, one may find fragments of an old rind included up to 3–4 element-diameters deep. And dark amber rind hyphae occur deep within the tubercle.

Rhizomorphs, concolorous with and having the same surface texture as the rind, extend from tubercles along roots and branch abundantly into the surrounding medium. Very young rhizomorphs connected to incipient tubercles are white to vinaceous. The mature rhizomorph is round in cross section, threadlike, up to 1 mm in diameter, and has a white core. The outer sheath is continuous with the rind, and the core enters and branches within the tubercle.

In long-wave (3660 Å) ultraviolet (uv.) light, the surface of the fresh, mature tubercle is a deep, velvety black and the interior is a moderately bright pink or rose.

Several chemical reagents listed by Singer (1962) were applied to tubercle tissues and color reactions noted. Viewed at × 12, concentrated NH_4OH and 15% KOH gave positive and similar results. Applied to shreds of rind, both immediately turned hyphae along tears from brown to green; body of shred became a darker brown-black with or without a green tint at first. When these two reagents were applied to interiors of fresh tubercles, mycorrhiza mantles and surrounding mycelium immediately turned a light pink. In older but still living tubercles with white interiors, a brown color developed. Reagents which gave negative or erratic reactions were guaiacol, phenol, formaldehyde, sulfovanillin, chlorovanillin, alpha-naphthol, pyrogallol, ferric sulfate, concentrated sulfuric acid, Melzer solution, and sulfobenzaldehyde.

Anatomy

For microtomed and stained sections, mycorrhizal elements were fixed in CRAF (Johansen 1940) and embedded in paraffin. Sections were cut 8–12 μ thick and stained with safranin followed by fast green.

Details of mantle structure were determined from fragments scraped from mycorrhiza elements using a small, sharp scalpel. For temporary use, these were mounted in polyvinyl alcohol (15 g dissolved in 100 ml of water) or 5% KOH. Permanent mounts were made with Hoyer's medium (Anderson 1954).

The rind of the mature tubercle, that part which can be peeled off easily, is 10–60 μ, mostly about 25 μ thick. Felt-like, it consists of densely interwoven, amber, aseptate, and thick-walled hyphae. Spores observed within the rind by Trappe (1965) were not found in any of the specimens examined. Hyphae are regular in diameter, from 1.5–4.5 μ, mostly about 2.5 μ and straight to curved. Walls are clean to heavily encrusted with golden-yellow amorphous deposits and are highly refractive in phase-contrast light. Along the inner surface of the tubercle rind, these hyphae attach to hyaline, septate, and thin-walled hyphae which extend as a loose mycelial mass and connect to mycorrhiza mantles. However, the actual juncture of the two hyphae is difficult to observe. In either sectioned or teased-apart material, they appear unconnected and unrelated and are easily mistakenly interpreted as belonging to different species.

Slight mechanical stress, such as a gentle teasing apart of the inner layer of the tubercle rind, tears away the weaker thin-walled hypha. Usually the break is clean leaving no remnant, but occasionally a short stub of the septate hypha remains attached to the aseptate hypha; only rarely can one find a longer length with septa attached. The septate hypha is transformed along its axis to an aseptate one, separated by a cross wall and occasionally by a clamp connection. Or, more commonly, it terminates in a sickle-shaped aseptate hypha. This characteristic

FIG. 1. *Pseudotsuga menziesii* + *Rhizopogon vinicolor* mycorrhiza. A. Tubercles and rhizomorphs along root taken from top of large decayed stump. Pinnate mycorrhiza is unidentified (× 2.0). B. Tubercle rind partially removed exposing tightly packed elements within (× 3.5). C. Opened small tubercle showing pinnate arrangement of elements (× 5.0).

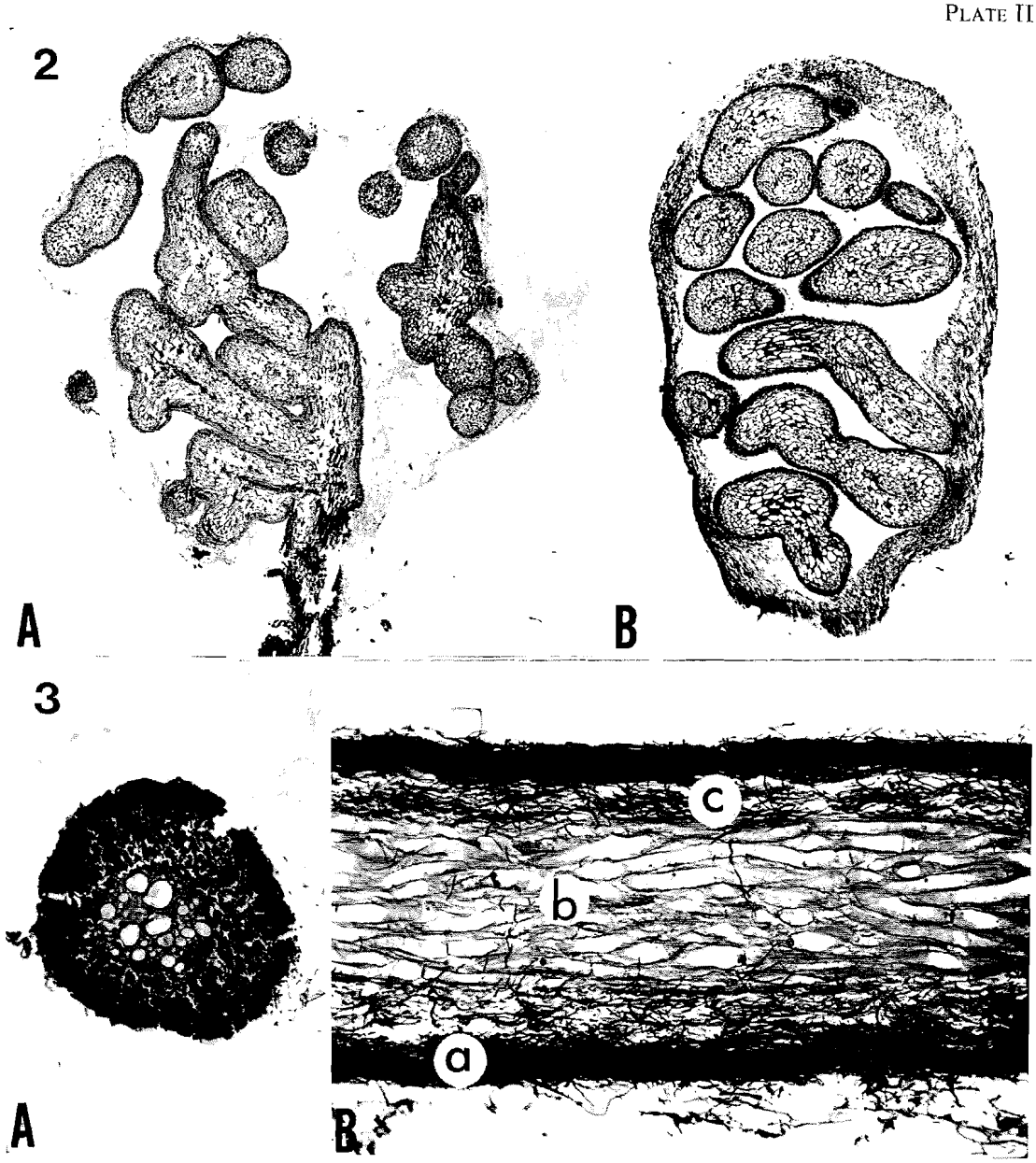


FIG. 2. Sectioned tubercles showing internal structure. A. Young enlarging tubercle covered by thin mycelial veil containing few amber aseptate hyphae (× 15). B. Mature tubercle with thick rind made up almost wholly of amber aseptate hyphae (× 20).

FIG. 3. *Pseudotsuga menziesii* + *Rhizopogon vinicolor* mycorrhiza rhizomorph. A. Cross section of small rhizomorph with core resembling vascular bundle (× 134). B. Longitudinal section of larger rhizomorph showing sheath (*a*) of amber aseptate hyphae, core (*b*) of large-diameter conducting hyphae, and inner ring (*c*) of amber aseptate hyphae and hyaline septate hyphae (× 134).

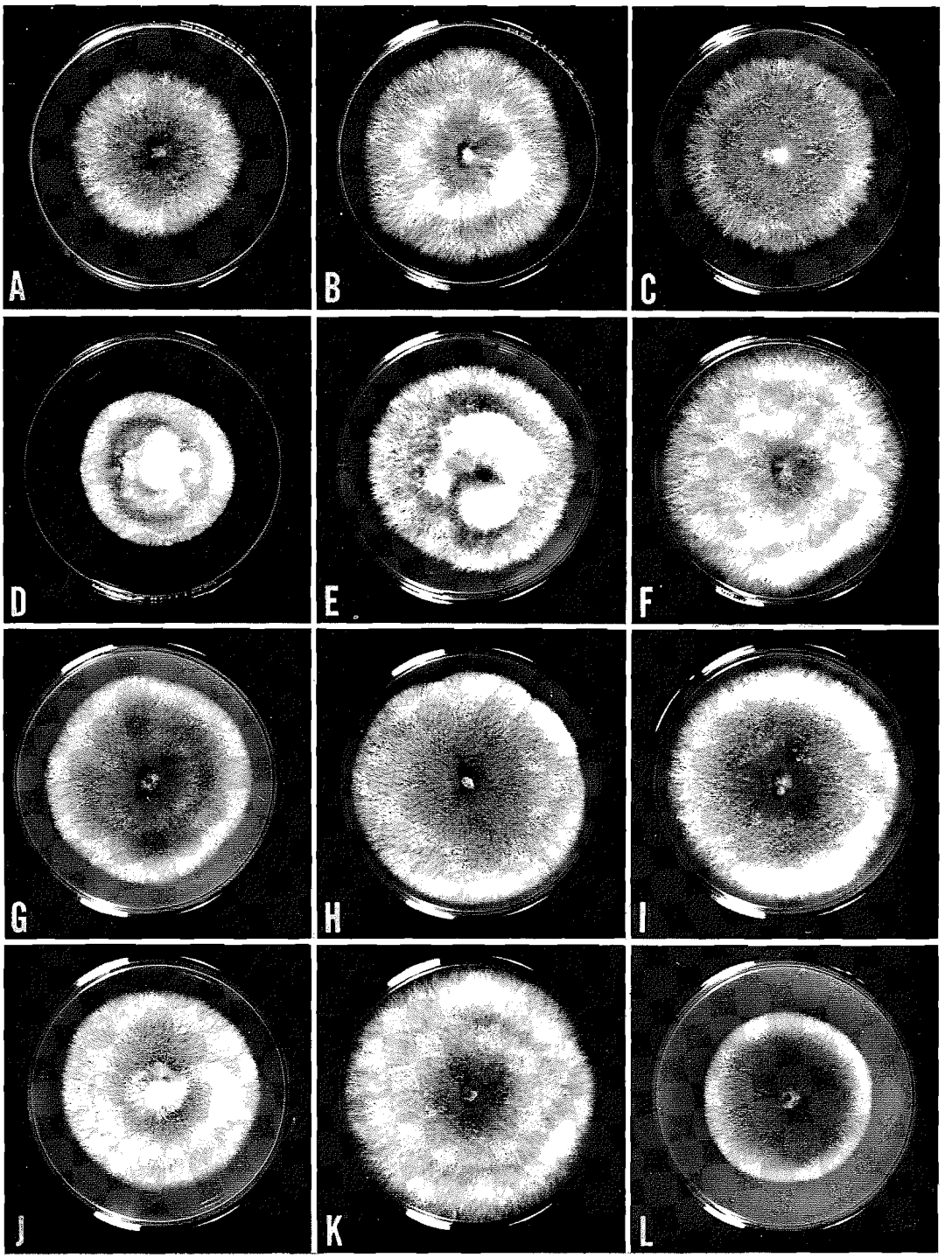
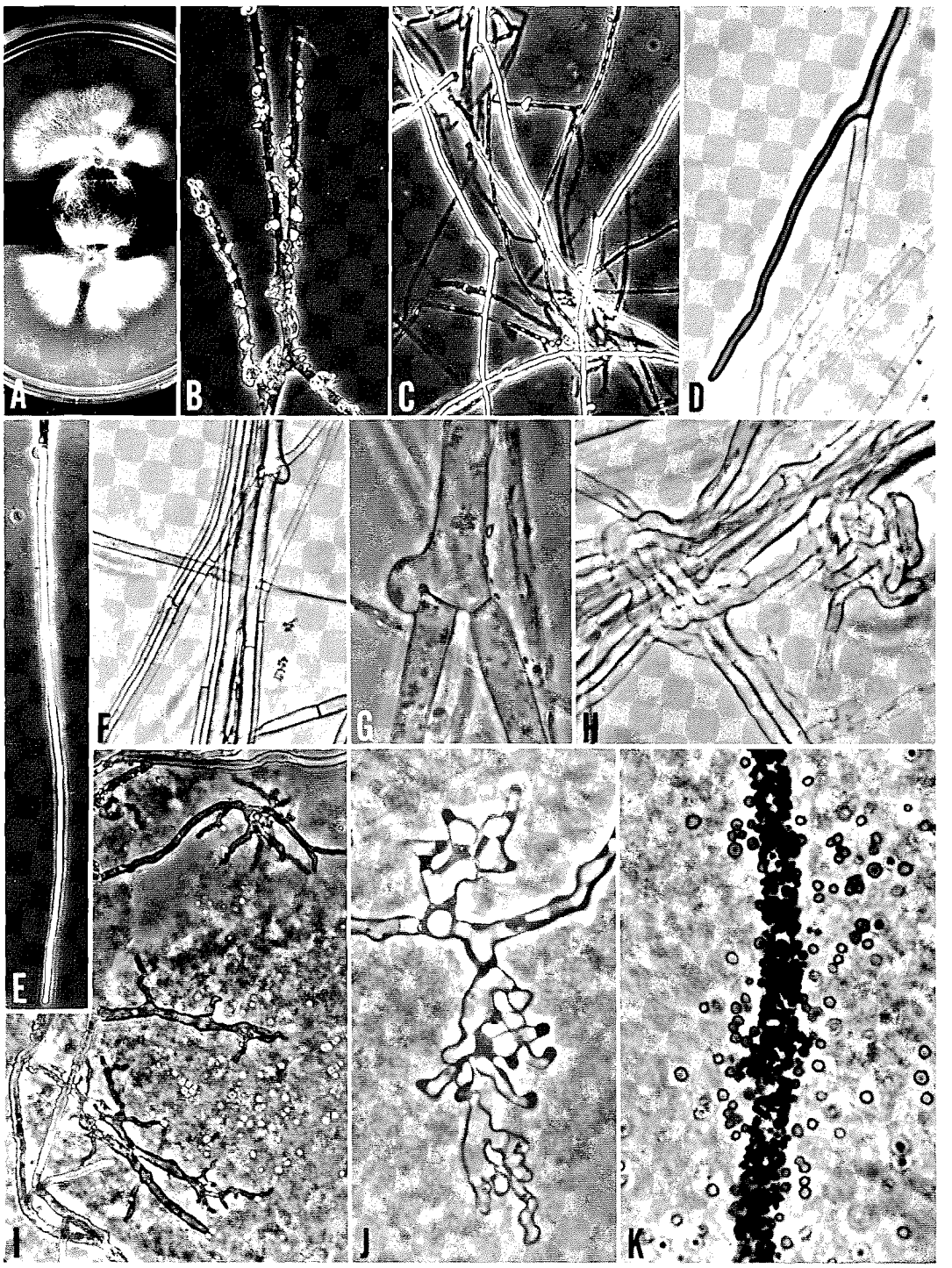


FIG. 4. Mats of different isolates of *Rhizopogon vinicolor* on MMN (A–F) and PDA (G–L) after 20 days in the dark at 20°C. Pairs A, G; B, H; and C, I are three different sporocarpic isolates and pairs D, J; E, K; and F, L three different mycorrhiza isolates.


A
B
C
D
E
F
G
H
I
J
K

sickle-shaped hypha may also occur within the tubercle rind. Some of these features are more fully detailed later in characterizing aerial and submerged mycelium of the fungal symbiont grown on nutrient agar medium.

Septate, hyaline hyphae, extending from the inner surface of the rind and connecting in a loose mycelial mass to mantle surfaces, are mostly straight and regular in diameter (1.5–5.5 μ, mostly 2–4 μ). They are thin-walled, lightly to heavily encrusted with hyaline amorphous deposits, have simple branching, numerous "H" fusions, and few clamp connections. Interspersed among these are large-diameter (up to 30 μ) hyphae extending from large, conducting core hyphae of rhizomorphs.

The internal elements are typically ectomycorrhizal. Except for a loose, tangled surface layer of hyphae, the 10- to 40-μ thick mantle consists of a dense layer of interwoven hyphae. These are hyaline, septate, thin-walled, and regular in diameter from 2 to 7 μ, mostly about 3 to 4 μ. A labyrinthine pattern is formed in contact with walls of first cortical cells. Usually a tier of distorted and discolored cortical cells is included in the inner part of the mantle.

The well-formed Hartig net penetrates four or five cortical-cell layers deep, almost to the endodermis. Separation between cells is usually one hyphal thickness, about 2–3 μ, but may be more in the outer tier of cells. Contact with cell walls is in labyrinthine pattern.

The mature rhizomorph consists of an outer sheath of aseptate hyphae, a ring of a mixture of aseptate and septate hyphae, and a core of large-diameter, conducting hyphae (Fig. 3A, B). In a rhizomorph 250-μ in diameter the sheath may be 10–20 μ and the inner ring 40–50 μ thick and the core 110–150 μ in diameter. The structure and hyphae of the sheath and inner ring are similar to those of the rind and inner loose mycelium of the tubercle. The core, however, has several to as many as 30, depending on rhizomorph thickness, large-diameter (up to 40 μ), septate, and thin-walled hyphae. These are tightly surrounded by small-diameter (2–4 μ) hyphae which occasionally bear clamp connections. In cross section (Fig. 3A) a small core resembles a plant stem vascular bundle. Within the tubercle, the core branches into silvery-white, loose strands from which small-diameter hyphae ramify and connect to element mantles.

Isolation of Fungus

The fungal symbiont, *Rhizopogon vinicolor*, is one of the easiest to isolate from an ectomycorrhiza. With the following procedure and fresh tubercles, isolation success of 80–100% was obtained.

After the tubercle was washed and dried, the rind was peeled away and the internal elements pulled apart. Pieces of elements were cut 2–5 mm long and immersed in 30% H_2O_2 for 5–20 s followed immediately by a 2- to 5-min wash in sterile water (Zak 1969*a*). They were then plated on potato–dextrose agar medium formulated by Lacy and Bridgmon (1962) (hereafter designated PDA) or on Melin-Norkrans medium modified by Marx (1969) (hereafter designated MMN).

Cultural Characteristics of Fungi

Macro- and micro-scopic characteristics of the fungal symbiont were determined from nutrient agar cultures of eight isolates, five from mycorrhizae and three from sporocarps (*Trappe 396*, *705*, and *801*, OSC). Isolates were grown on PDA and MMN media, 5 mm deep, pH 5.5 (±0.2), in petri dishes 100 mm in diameter at 20°C in the dark for 20 days. Also, microscopic features were determined from undisturbed mycelial growth induced on glass cover slips from PDA. Cover slips were permanently mounted on slides with Hoyer's medium (Anderson 1954).

Macroscopic Characteristics

One-week MMN mat was raised, cottony, and white; 20-day mat (Fig. 4A–F), raised 0.1–1.0 mm at even to occasionally indented margin to 5 mm in center, center chamois-like

FIG. 5. Microscopic characteristics of *Rhizopogon vinicolor* grown on PDA. A. Aerial mycelium induced on glass cover slip for microscopic study. B–H. Aerial mycelium. B. Encrusted hyphae. C. Teased-apart mycelium including aseptate hyphae with characteristic crook like those of tubercle rind. Note remaining stubs of thin-walled septate hyphae. D. Aseptate hypha with crook, formed from attached thin-walled septate hypha. E. Clamp connection between septate and aseptate hyphae. F. Hyphal strands. G. Clamp connection. H. Slender hyphae of strands forming laybrinthine design on glass similar to Hartig net. I–K. Submerged mycelium. I. Regular-diameter hyphae giving rise to dendritic hyphae. J. Dendritic hyphae. K. Spherical to irregular purple deposits along hyphal wall; those in surrounding agar are spherical. Lighting of B–K is phase contrast. B, C, E, F, I, and K are × 405; D, G, H, and J are × 1010.

to felty to floccose to cottony, marginal zone felty to cottony, center cream to tan to light chocolate brown, margin tan to white, concentric banding only slightly apparent; medium unstained along margin; underside mat deep brown-black to black with narrow light brown to cream margin.

One-week and 20-day PDA mats similar to MMN mats with following exceptions: 20-day PDA mat (Fig. 4G–L) raised 0.5–3.0 mm in center; center from light chocolate brown to light tan to white, rest light brown, mats of some isolates with pronounced concentric bands, as many as eight, of light brown, dull yellow, and light tan to white; medium along margin for 0.5–1.0 cm stained light brown to dull yellow; underside mat deep brown-black with narrow light brown or dull yellow margin.

Mats of different isolates displayed much variation in texture, color, and 20-day diameter on both media; diameter growth on PDA 6.0–9.0 cm (7.5 cm average) and on MMN 3.9–8.3 cm (6.6 cm average).

PDA and MMN mat 3660 Å uv.-light fluorescence variable among isolates; intensity moderate; generally entire mat dark wine red, or only center, with rest lighter hue; concentric rings, when present, fluoresce as alternate bands of dark and light wine red.

Of chemical reagents tested on tubercle tissues, only concentrated NH_4OH and 15% KOH gave positive and useful reactions, with little variation among different isolates, on 20-day PDA and MMN mats; concentrated NH_4OH fumes immediately turned aerial mycelium from white or light tan to pink, drop on mat center produced sudden "explosive" darkening outward to margin beneath mat, and drop on agar at margin turned hyaline submerged mycelium dull green within $\frac{1}{2}$ min; drop 15% KOH on white or light tan aerial mycelium turned it dull pink to red to pinkish-brown within 1–2 min, and drop on agar at margin turned submerged mycelium weakly to strongly dull blue-green to olive green almost immediately, latter reaction more positive than with concentrated NH_4OH.

Microscopic Characteristics

A. Aerial Hyphae

Young, white mat bears only septate, hyaline, and thin-walled hyphae; 20-day mat has mixture of these, scattered aseptate, very thin-walled, fluid-containing hyphae, and aseptate, amber, thick-walled hyphae closely resembling aseptate hyphae of tubercle rind and rhizomorph sheath.

Young hyphal strands of undisturbed mycelium on glass cover slip (Fig. 5A) have one or two large-diameter (3–7 μ, mostly 4–5 μ), septate, hyaline, moderately thick-walled hyphae with frequent clamp connections (Fig. 5F–G); simple or paired branching gives rise to another same hypha or to small-diameter (1.5–3 μ, mostly about 2 μ), septate, hyaline hypha with thin walls and frequent clamp connections and "H" fusions, several to many lying parallel alongside larger hyphae; simple branching produces another same small hypha which may parallel strand or diverge, some occasionally bearing terminal vesicle, some forming labyrinthine design on glass surface (Fig. 5H), and some giving rise to aseptate, amber, thick-walled hyphae like those of tubercle rind, with or without characteristic sickle crook (Fig. 5C–E).

Older strands similar but with more hyphae, many becoming amber, thicker walled, lightly to moderately encrusted with amber amorphous deposits (Fig. 5B), and highly refractive in phase contrast light.

B. Submerged Hyphae

Septate, mostly thin-walled, straight, regular diameter (2.5–7.5 μ, mostly 3–5 μ), branching simple, clamp connections frequent, no hyphal fusions, few to many terminal and intercalary vesicles (up to 20 μ in diameter), hyaline to pale yellow (from yellow spicules along walls), few amber and deep purple, and few highly refractive in phase contrast light; many hyaline hyphae covered with spherical to irregular purple deposits (Fig. 5K) immediately turning blue-green in 5% KOH, also free in agar around hyphae but largely spherical and up to 7 μ in diameter; these hyphae give rise to septate, thin-walled, straight, regular but small-diameter (1–2.5 μ, mostly 1.5–1.8 μ), opaque (in 5% KOH) hyphae lacking clamp connections or hyphal fusions, and to septate, very thin-walled, variable-diameter (1–5 μ), dendritic hyphae (Fig. 5I–J), both occasionally lightly encrusted with purple deposits.

Wall incrustations of tubercle and cultured mat hyphae only slightly dissolved away in 5% KOH or saturated chloral hydrate.

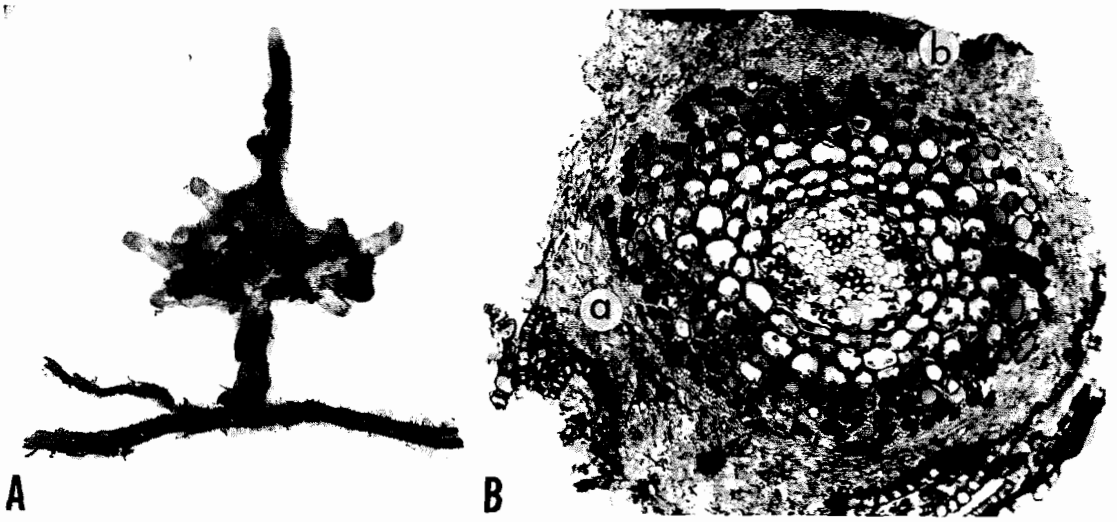
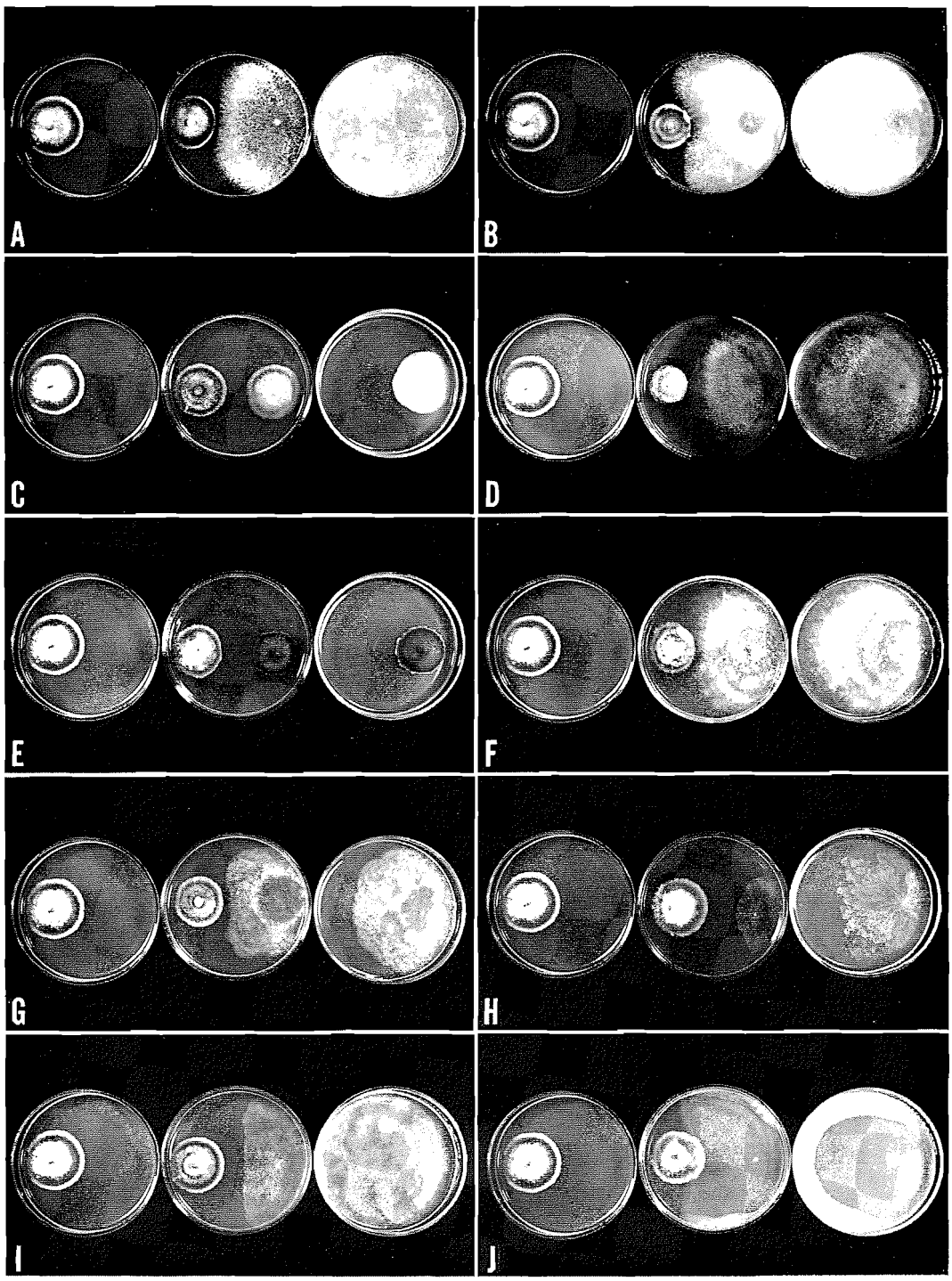


FIG. 6. Pure culture synthesis of mycorrhizae with Douglas-fir seedlings and *Rhizopogon vinicolor*. A. Pinnate mycorrhiza formed after 4 months. Tubercle did not develop but surfaces irregularly covered by patches of dark brown mycelium (× 3.7). B. Cross section of mycorrhizal element. Mantle (*a*) is thick and irregular; dark, superficial mycelial patch (*b*) is composed of same amber aseptate hyphae as tubercle rind (× 100).


A
B
C
D
E
F
G
H
I
J

Evidence of Association

Identification of the fungal symbiont of the Douglas-fir tuberculate mycorrhiza as *Rhizopogon vinicolor* is based on several criteria. First, sporocarps of *R. vinicolor* were found in close proximity to tubercles in large, decayed stumps.

Second, PDA and MMN cultures of three sporocarp and five mycorrhiza isolates were examined macro- and micro-scopically and found to be the same fungus, although variation in diameter growth, texture, and color of mat was rather high within each source group.

Third, uv.-fluorescence (3660 Å) of PDA and MMN mats of sporocarp isolates was identical with that of mats of mycorrhiza isolates.

Fourth, concentrated NH_4OH and 15% KOH applied to PDA and MMN mats produced the same color reactions for both sporocarp and mycorrhiza isolates. Also, these reagents induced similar colors with rind and mantle tissues of mycorrhiza tubercles.

Fifth, although structures resembling the natural tubercle failed to develop in pure-culture mycorrhiza synthesis with Douglas-fir seedlings, isolates of the fungus from both groups formed closely similar pinnate mycorrhizae (Fig. 6A–B). The rind of the natural tubercle was absent, but the white mantle surfaces were lightly coated with patches of loose brown mycelium containing aseptate, amber hyphae identical with those of the tubercle rind.

Rhizopogon vinicolor versus Root Pathogens

Antagonism by *Rhizopogon vinicolor* to 10 root pathogens was tested in triplicate on MMN medium in petri dishes. Inoculum of a mycorrhiza isolate of *R. vinicolor* was placed 2 cm from the edge of the dish and incubated in the dark at 20°C. Fifteen days later, pathogen inoculum was introduced 2 cm from the opposite edge. Figure 7A–J shows dishes, together with respective fungal symbiont and pathogen controls, 5–10 days (variable according to rate of growth of individual pathogen) after pathogen inoculation. *Phytophthora cinnamomi* Rands (Fig. 7G), *Pythium debaryanum* Heese (Fig. 7H), and *Pythium sylvaticum* Campbell and Hendrix (Fig. 7I) were strongly inhibited, indicating possible antibiotic action; *Fomes annosus* (Fr.) Cke. (Fig. 7A) and *Poria weirii* Murr. (Fig. 7B) were moderately inhibited; and *Fusarium oxysporum* f. *pini* (Hartig) Snyd. and Hans. (Fig. 7C), *Mycelium radicis atrovirens* Melin (Fig. 7E), *Pythium ultimum* Trow. (Fig. 7J), *Rhizoctonia solani* Kuehn (Fig. 7F), and *Macrophomina phaseoli* (Maubl.) (Fig. 7D) were weakly or not inhibited.

Discussion

According to Trappe (1962), several other species of *Rhizopogon* have been associated with mycorrhizae of conifers. *R. luteolus* Fr. & Nordh., *R. parasiticus* Coker & Totten, *R. roseolus* (Corda) Hollós, and *R. rubescens* Tul. have been linked to various species of pine. *R. rubescens* is also believed to be mycorrhizal with roots of Douglas fir and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.). The closely related *Truncocolumella citrina* Zeller is listed as mycorrhizal with Douglas fir. Pure culture synthesis of seedling Douglas-fir mycorrhizae with *R. colossus* A. H. Smith was reported by Trappe (1967).

Hatch (1937) concluded that part of the benefit of improved absorption of nutrients from the soil by the ectomycorrhiza may be attributed to the larger surface area of the mycorrhizal in contrast to the nonmycorrhizal root. Although surface area of mycorrhiza elements within the Douglas-fir tubercle mycorrhiza is considerable, it is sealed off from contact with the soil. Trappe (1965) has suggested that for this mycorrhiza the widespread network of rhizomorphs may instead serve to absorb and conduct nutrients from substratum to tree root. The presence in the rhizomorph core of very large-diameter, conducting hyphae which ramify and terminate within the tubercles, connecting to mantle surfaces via smaller hyphae, lends support to this view. The mycorrhiza elements themselves may serve merely to collect and transfer nutrients moving into the tree.

FIG. 7. Mycorrhizal isolate of *Rhizopogon vinicolor* versus root pathogens in the dark at 20°C. *R. vinicolor* mat was 15 days old when pathogen inoculum added. Photographs were made 5–10 days (depending upon rate of growth of individual pathogens) later. In each series, *R. vinicolor* control is in left dish, pathogen control in right dish, and both together in center dish. Pathogens tested are (A) *Fomes annosus*; (B) *Poria weirii*; (C) *Fusarium oxysporum* f. *pini*; (D) *Macrophomina phaseoli*; (E) *Mycelium radicis atrovirens*; (F) *Rhizoctonia solani*; (G) *Phytophthora cinnamomi*; (H) *Pythium debaryanum*; (I) *Pythium sylvaticum*; (J) *Pythium ultimum*.

Results of antagonism tests between *Rhizopogon vinicolor* and root pathogens conducted on nutrient agar medium can only be regarded as suggestive relative to natural conditions. However, it is possible that, besides aiding nutrition of the tree, the tuberculate mycorrhiza serves to ward off pathogen attack by one or more of the mechanisms discussed by Zak (1964). Secretion of an antibiotic, as Marx and Davey (1969) clearly demonstrated with *Pinus echinata* + *Leucopaxillus cerealis* var. *piceina*[2] mycorrhizae, may be one. And, unlike other forms, the tuberculate mycorrhiza is unique in the additional protection of a thick and dense fungal rind completely encasing mycorrhiza elements. It should at least serve as an effective mechanical barrier to pathogen entrance and especially to penetration by zoospores of injurious Phycomycetes.

The tubercle rind also probably protects internal tissues from aphid attack. Colonies of *Rhizomaria piceae* Hartig (referred to as "probably *Pemphigus piceae* Htg." by Zak 1965) are common, especially in decayed wood, feeding on several forms of mycorrhizae in Douglas-fir stands in western Oregon. However, they have never been observed attacking *Pseudotsuga menziesii* + *Rhizopogon vinicolor* tubercles despite heavy infestation of other mycorrhizae close by and along the same root. Thickness of the rind probably prevents insertion of their stylus into cortical and vascular tissues.

Acknowledgment

I thank Mrs. Darlene Duff for preparation of microtomed sections.

ANDERSON, L. E. 1954. Hoyer's solution as a rapid permanent mounting medium for bryophytes. Bryologist, **57**: 242–244.

DOMINIK, T. 1963. Occurrence of Douglas-fir (*Pseudotsuga taxifolia* Britton) in various Polish stands. Prace Inst. Badaw. Leśn. **258**: 29–59. (U.S. Dep. Commerce TT 65-50353. 1966.)

DOMINIK, T., and I. MAJCHROWICZ. 1967. Studies on the tuberculate mycorrhizae of Douglas-fir (*Pseudotsuga taxifolia* Britton). Ekol. Pol. Ser. A, **15**(3): 75–90.

HATCH, A. B. 1937. The physical basis of mycotrophy in the genus *Pinus*. Black Rock Forest. Bull. No. 6.

JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., New York.

LACY, M. L., and G. H. BRIDGMON. 1962. Potato-dextrose agar prepared from dehydrated potatoes. Phytopathology, **52**: 173.

MARX, D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology, **59**: 153–163.

MARX, D. H., and C. B. DAVEY. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. III. Resistance of aseptically formed mycorrhizae to infection by *Phytophthora cinnamomi*. Phytopathology, **59**: 549–558.

SINGER, R. 1962. The Agaricales in modern taxonomy. 2nd ed. J. Cramer, Weinheim.

TRAPPE, J. M. 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Rev., Oct.–Dec.: 538–606.

——— 1965. Tuberculate mycorrhizae of Douglas-fir. Forest Sci. **11**: 27–32.

——— 1967. Pure culture synthesis of Douglas-fir mycorrhizae with species of *Hebeloma*, *Suillus*, *Rhizopogon*, and *Astraeus*. Forest Sci. **13**: 121–130.

ZAK, B. 1964. Role of mycorrhizae in root disease. Annu. Rev. Phytopathol. **2**: 377–392.

——— 1965. Aphids feeding on mycorrhizae of Douglas-fir. Forest Sci. **11**: 410–411.

——— 1969*a*. Characterization and classification of mycorrhizae of Douglas fir. I. *Pseudotsuga menziesii* + *Poria terrestris* (blue- and orange-staining strains). Can. J. Bot. **47**: 1833–1840.

——— 1969*b*. Four *Poria terrestris* (DC. ex Fries) Sacc. strains mycorrhizal with roots of Douglas-fir. Abstr. XI Int. Bot. Congr., Seattle, 1969. p. 247.

[2]*P. echinata* Mill.; *L. cerealis* var. *piceina* (Peck) ined.